

A REVIEW OF THE USES OF ENZYMES IN THE TANNERY*

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Abstract

For several centuries enzymes in the form of bates used in deliming have been part of the tanning process, and for the past seventy years research has been conducted on enzymes used for unhairing. These enzymes belong to a general classification known as proteolytic enzymes which catalyze the hydrolysis of a variety of different proteins. More recently, carbohydrases, which catalyze the breakdown of polysaccharides, and lipases, which hydrolyze fats into fatty acids and glycerol, have been proposed for the processing of hides and skins. These uses of enzymes have been the subject of a large amount of published literature from around the world. This review will summarize this research covering the period from 1965 to 1986 and is intended to provide some insight on the potential uses for enzymes in the tannery.

Introduction

For many years enzymes have played an important role in the tanning of hides and skins. American Indians took advantage of the naturally occurring enzymes in the hide to cause hair loosening by "sweating," a natural autolysis or breakdown process of non-living tissue. Sweating hides was a common method of unhairing at the turn of the century, and is still in limited use today particularly for sheepskins. These methods were, of course, difficult to control and generally resulted in damage to the grain. J. Turney Wood, in 1899, demonstrated that the effects observed in bating were in fact due to the enzymes produced by bacteria in dog dung or chicken manure infusions, in conjunction with weak acids or deliming salts (1). In 1908, Rohm prepared and standardized Oropon*** a bating material from pancreatic glands (2), and its successful promotion in this country was carried out by Haas, which led to the elimination of the dung bates. Shortly afterward, Rohm applied the knowledge he gained from the bating enzymes to the first successful enzymatic unhairing, later known as the Arazym process (3). He found that the use of pancreatic enzymes for bating could be extended to unhairing if the skins were swollen first in dilute caustic soda followed by neutralization with sodium bicarbonate. After introduction of the process to this country in 1920 by Hollander at the ALCA meeting (4), a practical enzymatic procedure for unhairing was worked out and standardized by Turley (5). It was used for a time on a commercial scale but in the early thirties was replaced by less expensive methods.

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Quite a number of papers have been written on the potential uses of enzymes in the tanning industry. A comprehensive review of the literature on the uses of enzymes in unhairing was published by Green in 1952 (6), and several authors, for example, Hetzel (7) and Yates (8), have given short reviews on the progress of enzyme unhairing research covering the late sixties and early seventies. Trabitzsch discussed the possibilities of employing enzymes in the beamhouse with emphasis on the use of enzymes from microorganisms as opposed to those from animal organs, for they could better provide a regular economic flow of raw material as required (9).

This paper attempts to summarize the research which has been carried out since 1965, not only on unhairing enzymes but also on enzymes used in soaking, degreasing and offal treatment. Because of the familiarity with enzymes used in the bate, they will not be reviewed. The references cited herein were obtained primarily from Chemical Abstracts and JALCA abstracts, and the remainder from the individual publications. Most of the original papers and patents have been consulted. Those which were not available are referenced either with the Chemical Abstract volume and abstract number or with the Journal which cited the reference.

UNHAIRING ENZYMES

Enzymes used in unhairing are generally of the proteolytic type; these enzymes catalyze the breakdown of proteins. Their origin can be animal, such as bovine or porcine pancreas, bacterial, such as *Streptomyces fradiae*, fungal, such as *Aspergillus oryzae*, and plant, such as *Adenopus breviflorus*, recently studied by Adewoye of Nigeria (10, 11). The pH at which these enzymes are active ranges from acidic, in which pepsin and papain could be used, through neutral, for enzymes such as pancreatin and those from *Bacillus subtilis*, to alkaline, for enzymes such as Novo dewooling enzyme and the alkaline protease reported by Alexander at a recent ALCA meeting (12). The advantages of enzymic unhairing are a reduction of sulfide content in the effluent, recovery of hair which is of good quality, and elimination of the bate in the deliming step. Disadvantages are that the enzymic processes are more expensive than the conventional processes, they require careful control and, because of the effect on the structure of the hides and skins, they may require subsequent process changes.

The bulk of the abstracts cited dealt with proteolytic enzymes which the authors claimed were effective in removing hair from hides and skins. These enzymes were reportedly used at varying pH's and temperatures, and they required a much longer reaction time than the more conventional processes. Various modifications were made in pretreating the hides or skins either with bases or disulphide cleaving compounds which the authors claimed accelerated the process. Other authors, e.g., Thorstensen (13), Hannigan (14), and Andrews and Dempsey (15) recommended reliming to give a better product. Such a large number of enzyme unhairing systems are described in the literature that it would be impossible in this paper to mention all the contributions. An attempt will be made to show the progress in this area by citing the work of authors who have published a number of comprehensive papers.

Extensive research on enzyme depilation was conducted by Yates. He investigated suitable bactericides which would keep down the bacterial population over the required period and at the same time would not interfere with the action of the enzyme (16). He followed this process by the retardation of wool loosening after treatment of the skin with the

appropriate bactericide in the absence of the enzyme. Wool loosening was measured by depilation load, defined by Lennox in 1945 (17), and modified by Yates (16). He investigated 18 different bactericides, but the results were not conclusive. Sodium fluoride, phenyl mercuric nitrate, and merthiolate showed some potential, and these were later tested in the depilatory enzyme preparations.

In a subsequent publication, in which a number of commercially available enzymes were compared for their depilatory activity on sheepskin, Yates questioned the necessity of a bactericide when applied with a rapidly acting depilatory (18). In this paper he showed the significance of pretreating with a base, for it accelerated the unhairing time about 25%. He also found that it was not necessary to maintain the alkaline pH of the skin to retain the advantage of the presoak.

Yates described the structural changes, by histological examination, which took place in the wool follicle during depilation with either acetic acid, Pancrozyme C1A, and crystalline trypsin (19). Yates concluded that crystalline trypsin was unsatisfactory as a depilating agent and this was substantiated in a paper and patents by Donovan (20,21). Yates stated that an "ideal system would quickly loosen the attachment of the bulb to the papillae, it would quickly effect separation of the outer root sheath from the underlying structure and it would be able to cause sufficient cell destruction of the outer root sheath to permit easy withdrawal of the fiber." Later, Yates described a technique for estimating depilating activity by injecting the enzyme from the flesh side as close as possible to the follicle (22). Yates found that 70% of the total time involved in depilation is taken up with the enzyme reaching its site of action (8). In the final publication in this series, Yates concerned himself with the mechanism of the enzyme depilation process and concluded that the "only type of enzyme activity that can be identified with depilation is a proteolytic activity of a broad specificity of the endopeptidase type" (23). In this paper, he also gave a brief review of the literature from 1951 to 1972 on the preparation of leather from enzyme depilated hides and skins, and concluded that this leather is "satisfactory and that the minor problems which arise in processing can be overcome by small modification in existing tannery processes."

Alberto Simoncini of Italy and Minoru Kubota of Japan published a series of papers on the use of enzymes from *Bacillus subtilis* as unhairing agents. Simoncini's series concerned an enzyme from *Bacillus subtilis* var. *vellens*. The hides were pretreated with lime, treated with sodium carbonate and 1.5% enzyme at pH 9.0-10.0 for 24 hours, and then relimed (24). He found at that time that a conventional tannage gave a wrinkled non-uniform grain, which could be corrected if a pretanning operation was carried out to fix the grain to make it more resistant to the astringent tannins. He subsequently modified the process to avoid a loss of hide substance and loosening of the fiber structure (25). This modification included increasing the enzyme concentration to 2% and using 0.5% lime, dimethylamine sulfate and sodium hydroxide instead of sodium carbonate. In a further modification the author pretreated the hides with 0.75% sodium sulfide for three hours which reduced the unhairing time by one third (26). The author claimed that these pelts were similar to those pretreated with an alkaline solution. If one would be interested in the wool quality from this process, the author stated that electron microscopic examination showed a wool that appeared unchanged after treatment (27).

Simoncini published several papers on various methods for preparation and purification of the enzyme (28,29). He suggested that the best fermentable broth is prepared with corn steep liquor, lactose, sodium sulfate, and potassium acid phosphate; and that the best yield is obtained when the starting pH is 6.8-7.2 at 37°C. for a period of growth of 96 hours.

Purification, which noticeably increased its activity, was carried out by either fractional precipitation with ammonium sulfate, fractional precipitation with protamine sulfate, or chromatographic separation on a cellulose column.

Simoncini also evaluated the activity of various enzymes for their unhairing activity (30). He found that pepsin, β -chymotrypsin and elastase were inactive; that trypsin and α -chymotrypsin were poor; and that Keratinase, Nercozyme 150, Arazym, Pronase, Napase, Biopraser, and Protease 306 were highly active.

In the first paper in Kubota's series, he discussed the optimal conditions of unhairing by spitase, an enzyme from *Bacillus subtilis* var. *biotecus* (31). The author stated that a pH of 8.8-9.3 and a temperature of 20-40°C for 24 hours was effective. In further studies on depilatory conditions, the author found that wet salted calfskin was unhaird using 0.5% of the commercial enzyme Biopraser SP-2 at pH of 9.0, but that fresh skins could be unhaird in this 24 hour time frame only if 1.5 to 3.0% lime were added (32). Practical experiments using this enzyme preparation required 1.0% of the commercial preparation, with 3.0% lime and 400% water (33). The resulting chrome-tanned and fat liquored products, when compared to conventional unhairing, gave a significantly greater elongation at break, but no other differences were found. In subsequent publications, the author described the unhairing activity of *Trametes sanguinea* at a pH less than 6.6 (34), and *Streptomyces griseus* at a pH of 9.0 (35), and found them both to be satisfactory.

Monsheimer and Pfeleiderer of Rohm, Darmstadt, were granted a number of patents on formulations for enzymatic unhairing of hides and skins. A patent was issued in 1970 which utilized pepsin and papain at pH 3.0-4.5 for unhairing (36). The authors claimed that by using a range of proteolytic enzymes, a single bath process for steeping, unhairing, and bating is possible. A major disadvantage would be the cost. However, the advantages would be that the process produced no keratin sludge or sulfide, a reduced BOD, and reduced water requirements. A patent was issued to the authors in 1975 (37) which was an update of several other patents which were issued in 1974 (38). The 1975 formulation included a combination of alkaline fungal and bacterial proteinases along with trypsin, thioglycolic acid and NaOH, and this was followed by a treatment with NaOH and lime. The authors claimed that the unhairing was completed in 7 hrs. Originally, in the patents in 1974, the authors included dimethylamine, mercaptoethanol, sodium sulfate, and/or sodium carbonate in their formula. In 1975, they published their enzymatic one-step process, which the authors claimed was their most important achievement (39). In 1977, Pfeleiderer published an update in which the formula could include small amounts of sodium sulfide and sodium sulfhydrate, and the process at this point is being referred to as the Erhazym process (40).

Recently, Pfeleiderer published an article in the British magazine *Leather* which describes auxiliary agents for the bovine tannery beamhouse (41). Among the various agents discussed is a formula for a low sulfide, one-step enzymatic process for unhairing. The author gives an estimation of wastewater quality with a subsequent reduction in sulfide, COD, BOD, and a reduction of hydrogen sulfide in deliming.

Several authors have made contributions to the understanding of how the enzymes affect the structure of their substrates. Andrews and Dempsey published several papers on the changes in fiber structure due to enzyme unhairing, stating that the fiber structure was ill-defined, and that the absence of plumping was reflected in the compact structure (42). In a second paper, they saw a splitting up of fiber bundles, but the weave pattern was compact. If the hides were limed, however, they became well opened up (15).

Herfield, in 1969, recommended an enzyme-lime-sulfide process (43). He stated that it

was not advisable to completely remove all the hair, especially the fine hair, by the enzyme process. Complete removal was possible, but the attack on the corium was so great that loose grain resulted, especially if no filling tannins or retannins were used. If at least 90% hair loosening was to be achieved, it was necessary to follow the enzyme bath with a lime sulfide treatment. This process not only loosened the hair, but also opened the fiber structure of the corium so much that a bate could not be used without harm.

In 1971 and 1973, Urbaniak reported on the influence of some enzyme preparations on the components of skin. He first dealt with noncollagenous proteins (44). Four enzyme preparations for unhairing, bating, or soaking were tested for a variety of enzymatic activities. The unhairing ability correlated with activity toward casein, and the bating ability with activity on elastin. A second paper dealt with collagenous proteins and skin tissue (45). The commercial preparations reduced the isometric tension developed by calfskin when heated in water. It was thought that the enzymes may act by reducing the number of intermolecular crosslinks leaving the collagen susceptible to further degradation under solubilizing conditions. This activity may provide a useful approach to the manufacture of gelatin or soluble collagens from trimmings, fleshings, and other residues. When the skin is tanned, new crosslinks are formed and satisfactory leather should result, provided no further damage to collagen occurs during subsequent processing.

Yates responded to the second paper and stated that there was an increase in solubility of the collagen before tanning when he unhaird with Novo Dewooling Enzyme #1 (46). He agreed that tanning appears to introduce intermolecular crosslinks that compensate for those broken in the initial enzyme treatment. The tanner must take into account that the enzyme dewooled stock is different from that to which he is accustomed.

Totev of Bulgaria wrote several papers on the effect of E-30, an unhairing enzyme, on bovine hides and sheepskins (47). The enzyme disorganized and almost totally destroyed the skin structure due to specific action of the enzyme preparation on the elastin fibers. The finished leathers were wrinkled, puckered, and creased. In a later publication, it was stated that treatment of the fresh hide with E-30 at pH 6.5-7.0 for one hour, followed by addition of 5% lime, to raise the pH to 12.5, improved the unhairing process and retained the elastin fiber network (48).

Istranov, in 1975, found that the action of relatively concentrated enzyme preparation, for example, protorhizin in 0.01M NaOH, on animal dermis had no effect on the physical structure of collagen fibers, but did increase their solubility in dilute acetic acid solution (49). The enzymes cleaved intermolecular bonds between the collagen fibrils and loosened their structure. Of course, some loosening is beneficial in leather processing. In 1978, Felicjaniak, of the Leather Research Institute, Lodz, Poland, stated the alkaline pretreatment of pigskins limited the destruction of the useable surface of the leather after enzyme depilation (50). Pancreatic enzymes, reacting on substrates modified by alkalization, caused only loosening of the surface structure of the dermis, but in non-alkalized skins, the enzymes damaged the skin grain with complete local destruction of the papillae. An added advantage of alkalization is that the skins were unhaird in one-third of the time.

Ti-Kang Chu of the Peoples Republic of China followed the path of the enzyme into the skin during enzymic hair removal (51). The enzymes entered into the skins only from the flesh side when the skins were unhaird at room temperature. When the skins were unhaird in warm alkali, the enzymes passed through both the grain and flesh surfaces, with the latter predominating slightly. The enzymes entering into the two surfaces gave different unhairing actions, and these unhairing actions also depended on the state of the raw skins, the pretreatments, and the unhairing methods. Enzymes which entered through

the flesh side were important in maintaining the quality of the leather. These findings were substantiated by Yuzhi Yang (52) who also found that in the early stages of unhairing, the amount and depth of enzymes entering from the flesh side were greater than those entering from the grain, but that in the intermediate and later stages, less enzymes were distributed near the flesh side than the grain side.

The development of unhairing methods that will reduce or eliminate the use of sulfide is high on the priority list of process improvements for modern tanners. Whether enzymic methods can fulfill this need will depend primarily on their cost and the speed at which they can be made to work. The optimal combination of enzymes which could effectively remove hair in the same time required by sulfide has not been found yet. Even if the right combination is found, current cost levels could be prohibitive. The production of low cost enzymes for this purpose may eventually become a reality as a spin off of the new biotechnology processes which make possible the mass production of single protein products such as enzymes.

SOAKING ENZYMES

Carbohydrases, which catalyze the breakdown of polysaccharides, and proteases are the types of enzymes proposed for use in the soaking of hides. Their origin can be either animal, bacterial or fungal. The pH optimum can range from 4.0-10.5. The advantages of an enzyme soak which will be described include shortening the wetting back time, loosening of the scud, initiation of the opening of the fiber structure, and, when used at an alkaline pH of less than 10.5, production of a product with less wrinkled grain. The major disadvantage of their use is the added expense.

In 1966, a patent by Otto Grimm described a soaking method which used proteolytic enzymes and carbohydrases at pH's of 5.5 to 10.0 (53). Enzymes from *Aspergillus parasiticus*, *A. flavus*, *A. oryzae*, and *Bacillus subtilis* were used alone or in mixtures. The resulting leathers were full, supple, and showed no loose grain. Trabitzsch also published papers in several journals (9,54) on the possibilities of using enzymes in the soak.

A patent from the Scientific Research Institute of the Fur Industry, USSR, was granted in 1966 for use of the carbohydrase enzyme of the mold culture *A. awamori* which allegedly improved the quality of the pelt (55). Rokhvarger, in a paper published in 1971 (56), used this enzyme at pH 4.5 to 5.0 at 30°C for 18 to 24 hours, and the author claimed that this improved the wetting of hides. In 1975, the author published a paper which suggested the use of rhizopine at 38°C, pH 4.0-5.0, for eight hours to soften woolskins (57). This treatment effectively removed 50% of the mucopolysaccharides, separated the bundles of collagen fibers, and generally softened the fur. A Hungarian patent was granted in 1972 which removed lipids and noncollagenous proteins by means of lipase and amylase of pancreatic origin (58). The hides were previously steeped, washed, and fleshed, and then were treated with the enzyme at pH of 5.0-6.5 at 38-41°C for three hours.

Toshev and Esaulenko wet back non-salted, preserved sheepskins with the enzymes of *A. oryzae* in 4 to 5 hrs (59). In a later paper, the authors described optimum conditions for extracting nitrogen containing components and carbohydrates by the use of the enzyme Bioferm and sodium bisulfite at pH 5.0 and 35°C (60). The authors found that by reducing the temperature to 25°C the extraction of monosaccharides was reduced markedly. They published yet another paper in which they found that the use of 0.5 to 0.6% of bacterial α -amylase for soaking dry, unsalted wool lambskins resulted in strong amylolytic activity.

much weaker proteolytic activity, and no lipolytic activity (61). It also was effective at a pH of 5.0 in the presence of sodium bisulfite.

Monsheimer and Pfeleiderer were also quite prolific in their research on soaking enzymes. In 1968, in *Das Leder*, Pfeleiderer discussed the advantages of using mold proteases at pH 5 or lower for soaking, unhairing and bating (62), and a patent appeared in 1970 which described soaking with pepsin and papain at a pH of 3.0-4.5 (36). In 1972, the authors were issued a patent in which they used an alkaline proteinase at 28°C for four hours on salted calfskin (63). Along this line, in 1974, they recommended soaking the hides in alkaline proteinases of bacterial and fungal origin and they claimed that this reduced the need for the liming chemical, by 30 to 60% (64). In 1981, they were issued two patents in which they proposed a formula for soaking hides, skins, and fur skins (65). The formula included dicyandiamide and a proteolytic enzyme at a pH of 10.5. After four hours the hides were uniformly soaked and showed no sticking of the fiber structure, whereas the fur skins were also well soaked with no evidence of hair slip. Pfeleiderer, in a recent issue of *Leather*, discussed the advantages of an enzyme soak (41).

Finally, in 1984, Konstantinov discussed the changes in the mucopolysaccharides in lambskin after treatment with pancreatic amylase (Bioferm) and bacterial α -amylase (66,67). The refreshment of fur lambskins ensured the degradation of the acid mucopolysaccharides of the interfibrillar substance and of the stabilizing structure of collagen, i.e. the mucoproteins and the mucoids. This treatment ensured a normal course of the subsequent technological processes.

With the use of more rapid processing methods in today's tanneries the use of enzymes in the soak for more rapid wetting may be a definite advantage, depending on the end product desired. As stated by Pfeleiderer (41) enzymes used at this stage could ensure a softer leather and a cleaner grain which would improve aniline leather. Again the cost of the application must be weighed against the improvement of quality.

DEGREASING ENZYMES

The type of enzymes proposed for use in degreasing are called lipases; they catalyze the breakdown of fats and can be animal, bacterial, fungal or plant in origin. The optimum pH ranges from 4.0 to 7.0. The advantages of using enzymes for degreasing are the elimination of solvents, reduction in surfactants and possible recovery of valuable by-products. The disadvantages are that the lipases do not remove all types of fats in the same way that solvents do and they add cost to the process.

In 1966, both Trabitzsch (9) and Tzicas (68) studied enzymes used on hides and mention the potential for lipases in degreasing skins. A Hungarian patent was issued in 1972 to Papp which described a formula in which animal skins were treated with lipase and amylase enzymes in the presence of deoxycholic acid catalyst to remove lipids and noncollagenous protein (58). The enzyme (pancreatin) was of animal origin. Toshev suggested in 1972 that the enzymes of *Aspergillus oryzae* eliminated the necessity of skin degreasing (59), and in 1972, a paper by Baldano was published which compared enzymatic and solvent degreasing of pigskin and stated that both these methods removed approximately 50% of the grease (69).

Studniarski of the Technical University of Poland, in Lodz, described a process whereby pigskins were unhaired by a very active enzyme preparation of great stability prepared by an irreversible precipitation of an enzyme from pork pancreas with boric acid and

acetone (70). This enzyme was primarily used to unhair pigskin, and it accomplished this in six hours when the treatment was preceded by an alkaline swelling of the pigskin with lime and an activation of the enzyme with ammonium sulfate and sodium sulfite. The author states that the subsequent degreasing of the skins was unnecessary since the lipolytic activity of the preparation reduced the grease content by approximately 80%.

In 1975, Jareckas of the USSR used a combination of two enzymes, Protosubtilin G2X and Lipavamorin G3X, for 18 hours at 39-40°C at a pH of 7.5 and succeeded in unhairing and degreasing pigskin (71). In a subsequent publication in 1976, in which Protofradin G3X was substituted for the Protosubtilin, the author stated that this treatment improved the texture of pigskin, removed fat, mucosaccharides and substances other than collagen (72). It was found that the structure became more open, retained more fat during fatliquoring, and produced more elastic and fuller leather than pigskin unhaird by more conventional preparations.

Yeshoda, of India, in 1978, published a paper in which she used fungal lipase at a pH of 3.2-3.6, 37°C, for one hour, and successfully degreased wool sheepskins (73). In a subsequent paper she degreased and bated simultaneously at a pH of 7.8-8.0 (74). She claimed that one advantage was the biological degradation of lipids in the effluent collection tanks.

In 1982 and in 1983, Muthukumaran of India published a series of papers on his studies concerning the degreasing of sheepskins with the acid lipase from *Rhizopus nodosus* (75-78). In the first paper he reported the data on the chemical, physical, and microscopic studies of suede garment leather degreased by this enzyme and compared the results with solvent degreasing. The author states that only minor differences were found. In the next paper, he described a technique of purification and characterization of the enzyme and found that the purified lipase was active at pH 5.8 and 40°C. The enzyme was purified to homogeneity by various chromatographic techniques, and the homogeneity of the enzyme was confirmed by polyacrylamide gel electrophoresis, immunodiffusion, and immunoelectrophoretic techniques. In a subsequent paper he studied the substrate specificity of the enzyme, and found that, compared to other alcohol esters, the enzyme hydrolyzed triglycerides to the greatest extent. The enzyme hydrolyzed positions 1 and 3 of the triglyceride indicating specificity for the hydrolysis of primary esters.

Zhang of the Peoples Republic of China used alkaline lipase in combination with the proteinase and pancreatin in softening pigskin to improve the degreasing effect (79). The author also combined degreasing with the unhairing.

However, in 1982, Vulliermet, publishing in *Technicuir*, stated that in her experiments using fungal lipase to degrease delimed and pickled sheepskins, the enzyme gave unsatisfactory results compared to the classical solvent method (80). In 1983, Pfeleiderer, publishing in *Das Leder*, stated that a combination of enzyme-compatible surfactants with the enzyme has a synergistic effect in both soaking and bating and thus ensures the optimum wet degreasing of rawhides, pelts, and wet blues (81). The use of surfactants alone results in less intensive emulsification and dispersion of the natural fat than that required for obtaining evenly dyed, largely unfinished, aniline leather. The author did not refer specifically to lipase as the degreasing enzyme. In 1984, a patent was issued, in which he degreased by bating the hides with proteolytic enzymes in the presence of surfactants (82). Moreover, in that same year, in a publication by Taeger and Pfeleiderer, the authors claimed that enzymatic degreasing with lipases was not yet recommended due to the cost and technical reasons (83).

Enzymatic processing of hides to facilitate grease removal obviously requires more study.

It appears that a combination of enzymes might be necessary because it is not just the breakdown of the grease that is needed but also the release of the grease from within the hide. As in the other potential areas for the use of enzymes in the tannery the cost factor will determine the extent of their use should they prove to be effective in degreasing.

OFFAL TREATMENT

Enzymes could be used in the treatment of fleshings and effluent from tannery processes. A combination of all the hydrolytic enzymes, including proteases, carbohydrases and lipases would be required. The advantages to be realized would include a protein by-product suitable for animal feed as well as energy conservation and fat recovery. Again, the major disadvantage would be the cost.

In 1968, a British patent, filed by Novo Laboratory, described the preparation of an enzyme which could be utilized, among other things, for treating sewage (84). The preparation contained at least one proteolytic enzyme of the serine type, produced by cultivation of a species of the genus *Bacillus* and exhibiting optimal proteolytic activity against hemoglobin in the presence of urea at a pH above 9.0.

Braeumer, Eckmayer, Monsheimer, and Pfleiderer in 1978 were issued a patent and subsequently published a paper on the enzymatic conversion of glue stock and other hide offal to technically usable products (85). The authors proposed pulverizing the hide wastes, hydrolyzing with an alkaline protease at a pH of 9.0-13.0 in the presence of urea, hydrolyzing with the enzyme at pH of 2.0-5.0 in the presence of a strong acid, and then separating the fat and albuminous hydrolyzate. In 1979, Bronowski, in *Das Leder*, described the enzymatic treatment of the offal obtained at fleshing machines (86). Treating fleshings with pancreatic enzymes instead of heat for the purpose of separating the fat from the proteinaceous matter required much less energy and increased the yield from 60-65% to over 90%. The author gave the optimum conditions for obtaining a suitable product for the chemical industry and for the manufacture of leather oils.

A recent abstract in *JALCA* describes large scale installations for the utilization of fleshings (87). The process consists of the enzymatic hydrolysis of the proteins, conditioning of the resulting liquid, separating the fat and solids present in the hydrolysate and providing storage facilities for them. The abstract states that the outstanding features of the process are a recovery of 91% of the fat in the fleshings and the ready acceptance of the hydrolysate by the city's sanitation department. It can also be applied as a fertilizer directly to the soil.

Finally, a recent publication from Yugoslavia describes the separation of fats from the fleshy wastes of cattlehide processing by treatment with and without enzymes (88). The authors claim that the total fat content was higher by separation without enzymes whereas the content of saponifiable fats was higher by separation with enzymes. No mention was made of conditions or of the type of enzyme used.

Offal treatment by enzymes seems to have potential as an economical method of waste treatment to generate by-products which could be useful to the chemical industry. Research in this area is quite timely. The potential exists not only for treatment of the fleshings but also for treatment of the offal from other stages of processing, e.g. liming and bluing.

Thorstensen, in *Practical Leather Technology*, has stated that the tanneries in the future will use a combination of chemical and enzymatic processes (13). The potential for use of enzymes in the tanning processes lies mainly in areas which could reduce or eliminate a variety of pollution problems by eliminating potentially dangerous chemicals from the

process, e.g. sulfide, and by conversion of waste products into potentially saleable by-products. Several technically feasible substitutions of enzymes into processing have been demonstrated. Whether they will eventually have an economic advantage is not as easily demonstrated. The major factors in their utilization will depend on lower costs for their application and the tradeoff between by-product value and the cost of disposal.

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DISCUSSION

Review of the Uses of Enzymes in the Tannery

by

M.M. TAYLOR AND DAVID BAILEY

MR. SATYENDRA DE (W.D. Byron and Sons, Discussion Leader): Thank you for this nice review paper. In view of the current environmental problems this gives us some thoughts as to how we might soak, unhair or degrease our hides and skins. You mentioned an enzyme degreasing system. Can you tell us what kind of enzymes are used in this system?

MISS TAYLOR: Theoretically they should be lipases. However, from the literature it appears that they may be a combination of lipolytic and proteolytic enzymes. I think more

research needs to be done in this area if there would be an interest in using this type of system for degreasing.

DR. K.T.W. ALEXANDER (BLMRA): Did you mention work on keratinase by Simoncini?

MISS TAYLOR: No, I mentioned keratinase in the abstract but after further researching I did not pursue that. Simoncini used an enzyme from *Bacillus subtilis* var *vellens*.

DR. ALEXANDER: Do you think there is a place for keratinases?

MISS TAYLOR: It is a possibility but it seems that after a patent was issued in the early 1960's, I found very little further work specifically using keratinase.